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ORIGINAL ARTICLE

Morphometric aspects of remodeling of the arterial bed of the testicles in post- resection portal and pulmonary hypertensions

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ABSTRACT

Aim: To perform a morphometric analysis of the features of vascular remodeling of the arterial bed of the testicles in post-resection portal and pulmonary hypertension.

Materials and Methods: The testes of 54 white rats were studied, which were divided into groups: 1st included 16 intact animals, 2nd – 20 rats with pulmonary hypertension, 3rd – 18 individuals with post-resection portal hypertension. Postresection pulmonary hypertension was modeled by right-sided pulmonectomy. Postresection portal hypertension was simulated by removing 58.1 % of the liver parenchyma.

Results: The outer diameter of the small-caliber arteries of the left testicle increased by 3.4% ($p<0.05$) in post-resection arterial pulmonary hypertension, and by 2.9% in post-resection portal hypertension. The inner diameter of the small-caliber arteries of the left testicle decreased by 7.7% ($p<0.001$) in pulmonary heart disease, and by 6.5% ($p<0.01$) in post-resection portal hypertension. The Kernogan index decreased by 23.0% ($p<0.001$), the Vogenvoort index increased by 1.26 times. In case of post-resection portal hypertension, the Kernogan index decreased by 19.0% ($p<0.001$) and the Wogenvoort's index increased by 1.19 times. The relative volume of damaged endotheliocytes in the small-caliber arteries of the left testis increased 20.6 times ($p<0.001$) in pulmonary heart disease, and increased 16.3 times ($p<0.001$) in post-resection portal hypertension.

Conclusions: Portal and pulmonary hypertension lead to pronounced remodeling of the arterial bed of the testicles, which is characterized by thickening of the arterial wall, narrowing of their lumen, significant changes in Wogenvoort and Kernogan indexes, atrophy, dystrophy, and necrobiosis of endotheliocytes.

KEY WORDS: testis, arterioles, pulmonary hypertension, portal hypertension

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INTRODUCTION

In recent decades, researchers increasingly pay attention to the peculiarities of the remodeling of the arterial bed of organs in various physiological and pathological conditions. It is worth noting that vascular remodeling means a change in their structure and function under various physiological and pathological conditions, i.e., it is the response of all components of the vascular wall (endotheliocytes, smooth muscle cells, connective tissue elements) to negative factors. The arterial bed of the organs, its full functioning, play an important role in their blood supply, provide adaptive and reserve capabilities in the conditions of pathology. When studying biological objects, morphologists increasingly use morphometric methods, which allow obtaining an objective quantitative morphological characteristic of the features of the structural rearrangement of organs and systems of the body during various physiological and pathological processes and logically interpreting them.

Studying the causes of infertility and ways to overcome it is an important and urgent problem of modern medical and biological science. In recent decades, indicators of men's reproductive and sexual health have been decreasing in many countries of the world, but in Ukraine they have a rapid and extremely negative trend. Reduced spermatogenic and hormonal function of the testicles can be provoked by acute and chronic circulatory disorders in the testicles and the body, endogenous intoxication that occurs in many pathological conditions, as well as the effects of physical and chemical environmental factors [1-5].

Currently, the pulmonary heart is gaining more and more importance due to the significant increase in chronic obstructive pulmonary diseases, chronic forms of tuberculosis, occupational lung injuries, in which the main cause of disability and mortality is the pulmonary heart. The pathogenesis of the latter is complex and quite multifaceted, which greatly complicates the adequate, timely diagnosis of pulmonary hypertension and pulmonary heart disease. Post-resection pulmonary arterial hypertension leads to structural rearrangement of the organs of the systemic circulation, abdominal cavity and pelvis, as well as remodeling of their structures. The testicles belong to organs whose hemodynamic disorders are complicated by various morphological changes in the vascular bed and their structures. It is worth noting that the peculiarities of the remodeling of testicular structures in post-resection pulmonary arterial hypertension have not been sufficiently studied [6-8].

Removal of large volumes of the liver, which are often carried out in modern surgical clinics, can lead to post-

resection portal hypertension. The latter can be complicated by bleeding from varicose veins of the esophagus, stomach, rectum, splenomegaly, ascites, secondary hypersplenism, parenchymal jaundice, portosystemic encephalopathy, multiple organ failure [9, 10, 5].

With portal hypertension, the hemodynamics in the system of the hepatic portal vein and the veins of the systemic circulation changes, which leads to difficulty in venous drainage from the organs and structural and functional changes in them. Long-term venous congestion, hypertension in the veins, their progression leads to the development of multiple organ failure. It is worth noting that changes in the structural elements of testicular arteries in post-resection portal hypertension have not been sufficiently studied.

AIM

The aim is to perform a morphometric analysis of the features of vascular remodeling of the arterial bed of the testicles in post-resection portal and pulmonary hypertension.

MATERIALS AND METHODS

The testicles of 54 white sexually mature male rats, which were divided into three groups, were studied using a complex of morphological methods. The 1st group included 16 intact animals, the 2nd – 20 rats with pulmonary hypertension, the 3rd – 18 experimental animals with post-resection portal hypertension. Postresection pulmonary hypertension was modeled by right-sided pulmonectomy. Post-resection portal hypertension in experimental animals was simulated by removing the left and right lateral lobes of the liver (58.1% of its parenchyma). Animals were euthanized by bloodletting under thiopental anesthesia one month after the start of the experiment. Pieces were cut from the testes, which were fixed in a 10% formalin solution, passed through ethyl alcohols of increasing concentration and placed in paraffin. Microtome sections after deparaffinization were stained with hematoxylin-eosin, according to the method of Weigert, Van Gieson, Mallory, Masson, toluidine blue [11, 12]. In the left and right testicles, the following were measured: outer diameter (ODLT, ODRT) and inner diameters (IDLT, IDRT) of small-caliber arteries, media thickness (MTLT, MTRT), Kernogan index (KILT, KIRT) and Wogenvoort index (WILT, WIRT), the height of endotheliocytes (HELT, HERT), the diameter of endotheliocyte nuclei (DENLT, DENRT), the nuclear-cytoplasmic ratio in endotheliocytes (NCRELT, NCRERT), the relative volume of damaged endotheliocytes (RVDELT, RVDERT)[4]. Quantitative indicators were processed statistically. The processing of the obtained results was carried out in the department of systematic statistical research of I. Horbachevsky Ternopil National Medical University in the STATISTIKA software package. The difference between comparative values was determined by the Student's t-test and Mann-Whitney U test [13]. Conducting experiments and euthanasia of experimental animals was carried out in compliance with the «General Ethical Principles of Animal Experiments» adopted by the First National Congress on Bioethics (Kyiv, 2001), in accordance with the «European

Table 1. Morphometric characteristics of small testicular arteries of experimental animals with post-resection pulmonary and portal hypertension (M±m)

Indicator	Observation group		
	1st	2nd	3rd
ODLT, μm	38,30±0,42	39,60±0,39*	39,40±0,33*
IDLT, μm	24,50±0,21	22,60±0,18**	22,90±0,18**
MTLT, μm	7,10±0,12	7,60±0,09**	7,45±0,09**
KILT, %	40,10±0,45	32,60±0,36***	33,70±0,36***
WILT, %	244.4±3,3	307,0±3,3***	290,6±3,6***
HELT, μm	6,10±0,09	6,08±0,08	6,08±0,08
DENLT, μm	3,12±0,03	3,09±0,03	3,11±0,03
NCRELT	0,262±0,003	0,266±0,003**	0,264±0,003**
RVDELT, %	2,10±0,02	43,28±0,05***	34,30±0,04***
ODRT, μm	37,57±0,42	38,76±0,36*	38,70±0,33*
IDRT, μm	24,52±0,21	23,10±0,21**	23,30±0,21**
MTRT, μm	7,06±0,12	7,40±0,12*	7,34±0,12*
KIRT, %	42,50±0,45	35,50±0,36***	36,20±0,33***
WIRT, %	235,1±3,9	281,5±3,6***	275,9±3,6***
HERT, μm	6,10±0,12	6,08±0,09	6,08±0,09
DENRT, μm	3,09±0,04	3,10±0,03	3,10±0,03
NCRERT	0,258±0,003	0,262±0,003**	0,260±0,003**
RVDERT, %	2,08±0,04	38 ,60±0,03***	32,90±0,03***

*Note. *- p<0.05; **– p<0.01; ***– p<0.001 compared to the 1st group of observations.*

Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes». as well as the Law of Ukraine «On the Protection of Animals from Cruelty» (dated February 21, 2006).

RESULTS

Morphometrically, in these experimental conditions, the small-caliber arteries of the left and right testicles of laboratory sexually mature white male rats were studied. The obtained results of the conducted research are presented in the Table 1.

A comprehensive analysis of the data in the specified table established that most of them changed significantly compared to the control indicators. Thus, the outer diameter of the small-caliber arteries of the left testicle in post-resection pulmonary arterial hypertension increased statistically significantly ($p<0.05$) by 3.4%, and in post-resection portal hypertension - by 2.9%. The thickness of the media of the specified vessels also increased markedly. In the 2nd observation group (post-resection pulmonary hypertension), the specified morphometric parameter with a high degree of reliability ($p<0.01$) increased by 7.0%, and in the 3rd group (post-resection portal hypertension) - by 4.9% ($p<0.01$).

The inner diameter of the small-caliber arteries of the left testicle with pulmonary heart decreased from (24.50±0.21) to (22.60±0.18) μm. The given morphometric parameters differed statistically significantly ($p<0.001$) from each other, and the last digital value was 7.7 % lower than the previous one. In the intact left testicle, the inner diameter of the studied vessels was equal to (24.50±0.21) μm, and in post-resection portal hypertension - (22.90±0.18) μm. A statistically significant difference was established between the given morphometric parameters ($p<0.01$). At the same time, the lumen of the small-caliber arteries of the left testicle in post-resection portal hypertension turned out to be 6.5% smaller compared to the control indicator. The Kernogan and Wogenvoort indexes of the studied vessels changed significantly under these experimental conditions. Thus, in case of post-resection arterial pulmonary hypertension, the specified morphometric indicator (Kernogan index) decreased by 23.0% ($p<0.001$), and in case of post-resection portal hypertension – by 19.0%. ($p<0.001$). The Wogenvoort index for pulmonary heart was equal to (307.0±3.3) %, and for portal hypertension – (290.6±3.6) %. The given morphometric parameters statistically significantly ($p<0.001$) exceeded the similar control quantitative morphological indicator by 1.26 and 1.19 times, respectively. It is worth noting that most researchers believe that the Kernogan and Wogenvoort indices adequately reflect the physiological capacity of arteries, and a decrease in the Kernogan index and an increase in the Wogenvoort index indicate a significant violation and deterioration of the arterial blood supply of the organ under study. The height of the endotheliocytes of the studied vessels and nuclei of the left testis in post-resection arterial pulmonary and portal hypertension changed little. At the same time, the nuclear-cytoplasmic index of the studied cells was almost unchanged, which indicated the stability of structural cellular homeostasis. In the 2nd group of observations, the height of endotheliocytes decreased by 0.33%, and the diameter of their nuclei by 0.97%, which led to unexpressed changes in the nuclear-cytoplasmic ratio in these cells. Under these experimental conditions, this morphometric parameter increased by 1.5% ($p<0.01$), which indicated a slight violation of structural cellular homeostasis. The relative volume of damaged endotheliocytes in the small-caliber arteries of the left testicle in pulmonary heart increased 20.6 times ($p<0.001$). In the 3rd group, the height of the endothelial cells of the studied vessels of the left testicle with post-resection portal hypertension changed slightly. At the same time, the specified quantitative morphological indicator decreased by only 0.33% ($p>0.05$). The diameters of the nuclei of the studied cells did not undergo any changes and were equal to (3.11±0.03) μm, while the indicator decreased by 0.3%. The nuclear-cytoplasmic ratio in the endotheliocytes of the small-caliber arteries of the left testicle in the 3rd group of observations exceeded the similar control indicator by only 0.8% ($p>0.01$), which indicated a slight violation of structural cellular homeostasis. The relative volume of damaged endotheliocytes in the small-caliber arteries of the left testicle in post-resection portal hypertension increased 16.3 times ($p<0.001$).

The morphometric parameters of the small-caliber arteries of the right testicle changed similarly, but to a lesser extent compared to the previous ones. Thus, the outer diameter of the studied vessels in post-resection arterial pulmonary hypertension increased statistically significantly ($p<0.05$) by 3.2%, and in post-resection portal hypertension - by 3.0%. At the same time, the thickness of the media increased by 4.8% ($p<0.05$) and 4.0% ($p<0.05$). The inner diameter of small-caliber arteries and the Kernogan index of the studied arteries of the right testicle decreased under the given conditions of the experiment. With pulmonary heart, the lumen of the studied vessels decreased by 6.1% ($p<0.01$), and with post-resection portal hypertension – by 5.2% ($p<0.001$). The Kernogan index under these experimental conditions was reduced by 19.7% and 17.4%, respectively ($p<0.001$), and the Wogenvoort index increased by 1.2 and 1.17 times and was equal to (281.5±3.6) % and (275.9±3.6) %.

The height of the endotheliocytes and the diameters of the nuclei of the small-caliber arteries of the right testicle in post-resection arterial pulmonary and portal hypertension changed slightly. The nuclear-cytoplasmic index of the studied cells was almost unchanged, which indicated the stability of structural cellular homeostasis. In the 2nd group of observations, the height of endotheliocytes decreased by 0.33%, and the diameter of their nuclei by 0.3%, which led to unexpressed changes in the nuclear-cytoplasmic ratio in these cells. Under these experimental conditions, this morphometric parameter increased by 1.5% ($p<0.01$), which indicated a slight violation of structural cellular homeostasis. In the 3rd group, the height of the endotheliocytes of the studied vessels of the right testicle with post-resection portal hypertension changed slightly. At the same time, the specified quantitative morphological indicator decreased by only 0.33% ($p>0.05$). At the same time, the diameters

of the nuclei of the studied cells were almost unchanged and were equal to (3.10±0.03) μm, while the indicator decreased by 0.3%. The nuclear-cytoplasmic ratio in the endotheliocytes of the small-caliber arteries of the right testicle in the 3rd group of observations exceeded the similar control indicator by only 0.8% ($p>0.01$), which indicated a slight violation of structural cellular homeostasis. The nuclear-cytoplasmic ratio in the endotheliocytes of the small-caliber arteries of the right testicle in the 3rd group of observations exceeded the similar control indicator by only 0.8% ($p>0.01$), which indicated a slight violation of structural cellular homeostasis.

This was also confirmed by the relative volume of damaged endotheliocytes in the studied arterial vessels of the right testicle. In case of post-resection pulmonary hypertension, the specified morphometric index in the right testicle increased by 18.6 times ($p<0.001$) compared to the similar control value, and in case of post-resection portal hypertension – by 15.8 times ($p<0.001$). The obtained data indicate that the remodeling of the testicular small-caliber arteries in post-resection pulmonary arterial hypertension was more pronounced compared to post-resection portal hypertension and dominated in the left testicle, which can be associated with the peculiarities of the venous outflow from it.

DISCUSSION

The conducted studies and the results obtained indicate that changes in haemodynamics that occur in post-resection portal and pulmonary hypertension lead to structural changes in the endothelial cells of the testicular arterial bed [3]. It is believed that the endothelium covering the inner surface of blood vessels is an important auto-, para- and endocrine organ with numerous regulatory functions. It is known that normally functioning endothelial cells produce nitric oxide (NO), which regulates vascular tone, affects vascular wall remodelling, determines the system of antioxidant defence and peroxidative aggression, inhibits platelet and macrophage aggregation and adhesion, and proliferation processes in the vascular wall. In addition to NO, other vasodilatory factors produced in the endothelium include prostacyclin, endothelial hyperpolarisation factor, C-type natriuretic peptide, etcIt is believed that the action of these factors becomes an important factor in the regulation of vascular tone with a decrease in NO [14]. Endothelial cell damage or activation disrupts normal regulatory mechanisms and leads to phenotypic changes (imbalance between relaxant and constrictor factors, anticoagulant and procoagulant mediators, promoters, and growth inhibitors) that are generally defined as endothelial dysfunction. In the arterial wall, certain consequences of endothelial dysfunction are directly related to the pathophysiology of atherosclerosis and are manifested by vascular reactivity and angiospasm; increased permeability to lipoproteins; dysregulation of vascular wall cell growth (decreased endothelial regeneration and increased proliferation of smooth muscle cells); shifts in haemostatic/fibrinolytic balance (promoting thrombin generation, platelet and fibrin deposition). When endotheliocytes are damaged, intercellular junctions are disrupted, which leads to increased permeability of endotheliocytes and disruption of their barrier function. Vascular permeability and dysfunction of the endothelial barrier are complicated by tissue swelling and contribute to increased extravasation of inflammatory elements into paravasal tissues and contribute to the chronicity of the pathological process [15].

Changes in small-caliber vessels were more pronounced compared to medium- and large-caliber arteries. These structural processes can be explained by the increased functional load of small vessels.

Light-optically, the narrowing of the lumen and the thickening of the wall of small arterial vessels were observed on the histological preparations of the testes, which were complicated by hypoxia, dystrophy, necrobiosis of the cells and tissues of the examined organ, and in the remote period to infiltrative sclerotic processes. Elastic structures in blood vessels with the phenomena of multiplication, fragmentation and destructionEndothelial cells with signs of edema, dystrophically and necrobiotically altered, sometimes desquamated. Foci of cellular infiltration and sclerosis were observed in the testicular stroma [9]. The revealed structural changes dominated in the left testicle and in conditions of post-resection arterial pulmonary hypertension. The predominance of morphological and morphometric changes in the vessels of the left testicle can be explained by the peculiarities of the venous outflow from this organ.

CONCLUSIONS

Post-resection portal and pulmonary hypertension leads to pronounced remodeling of the arterial bed of the testicles, which is characterized by thickening of the arterial wall, narrowing of their lumen, significant changes in Wogenvoort and Kernogan indices, atrophy, dystrophy and necrobiosis of endothelial cells, endothelial dysfunction, hypoxia, dystrophic-necrotic changes in cells and stromal structures, infiltration and sclerosis, damage to interstitial and endocrine components. The identified pathomorphological changes were dominant in the left testicle and pulmonary heart.

PROSPECTS FOR FURTHER RESEARCH

A comprehensive, detailed comprehensive study of the arterial bed of the testicles in post-resection portal and pulmonary hypertension will allow to significantly improve the diagnosis, correction and prevention of lesions of the specified organs in the pathology under study.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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